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Effect of solvents and cyclodextrin complexation on acid–base and photophysical properties of dapoxyl dye

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ABSTRACT

A pH dependent encapsulation of dapoxyl sodium sulfonate (DSS), a charge transfer (CT) dye, with α- and β-cyclodextrin (CD), macrocyclic molecules made up with sugar units, has been performed using steady-state and time-resolved fluorescence measurements; both confirm the formation of an efficient inclusion complex with both non-protonated and protonated form of DSS. Moreover, the fluorescence intensity is affected due to modulation of acid-dissociation constant by CD encapsulation as a consequence of preferential binding affinities with different prototopic forms of DSS. In particular, we observed that the relocation of DSS from water into the less polar hydrophobic cavity of CD causes huge fluorescence enhancement in water. Our investigation reveals that the micro-polarity inside β-CD cavity is akin to methanol–water (1:1) mixtures.

1. Introduction

Environment sensitive fluorescent dyes find a variety of applications in chemistry as well as in biology [1]. Probing the characteristics of micro-environments which can be very different from the bulk solution and utilization of such "new phase of matter" for stabilization of guest molecules by means of various experimental techniques have always been a constant interest and represented notable challenge among physical organic chemists [2]. Solvent-sensitive-solvatochromic molecules are key to probe the local polarity, polarizability, viscosity, and acidity [3]. In this regard, intramolecular charge transfer (ICT) fluorescent dye can be the perfect reporter molecules for investigating micro-environmental properties [3–8]. Typically an ICT dye molecule contains an electron rich moiety and an electron deficient group, and they are connected by a π-conjugated spacer commonly known as D-π-A type fluorophore [9]. An efficient charge transfer process from donor to the acceptor part of ICT dye in the ground state as well as in excited state results a high dipole moment [9]. As a consequence of this high dipole moment, the energy of the excited state can be altered through the interaction with surrounding solvent molecules. Dapoxyl sodium sulfonate (DSS, see Fig. 1) is a unique ICT dye in which an efficient charge transfer from dimethylaniline moiety to the benzene sulfonate part through oxazole ring and the acid dissociation constant is *ca.* 4.1 [10]. It exhibits an excellent response toward the change of local micro-environment and has indeed been employed for probing hydrophobic interior of a protein [10–15]. A series of DSS dye derivatives have already been synthesized to study their solvent-dependent optical properties, and quite a few of them having reactive functional group have been found to be potential for biological applications [11–15]. Fluorescence properties of DSS have been studied in various restricted environments *viz.* micelles, liposome and with β-CD [11,13]. Previous study on DSS and β-CD interaction was only performed at neutral pH and the role of pH of the surrounding medium was not explored. Two novel fluorescent derivatives of DSS have been synthesized to develop a ratiometric pH sensor using fluorescence [14]. Several other DSS dye derivatives with various functional groups have been made to label acidic organelles in live cells [15]. Dapoxyl sulfonamide has been used for Zn^{2+}ion biosensing with multiphoton excitation [16]. Large fluorescence enhancement of DSS by cucurbit[7]uril encapsulation followed by guest protonation has been elegantly exploited to monitor enzymatic activity *via* fluorescent-off assay, which is becoming an increasingly popular analytical method in supramolecular chemistry and biophysical chemistry [10,17–19].

Cyclodextrins (CDs, see Fig. 1) are widely explored naturally occurring macrocyclic host molecule with appreciable water solubility and non-toxicity [20]. CDs are made of glucose units with varying cavity sizes and the relative rigidity of the individual glucose unit makes them more special for molecular recognition and encapsulation [20]. Hydrophobic cavity along with hydrogen bonding possibility with polar and negatively charged guest molecule make CDs well-suited as nano-containers for their

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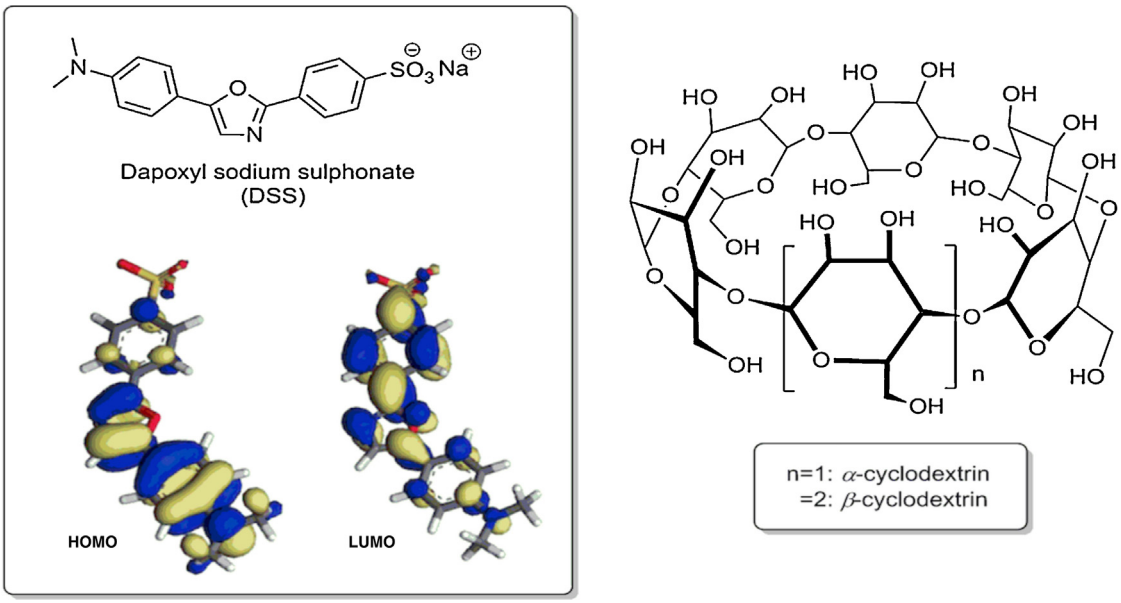

Fig. 1. Structure (top left) and graphical representation of HOMO and LUMO of DSS in water (bottom left) and structure of CD (right).

investigation. Depending on the number of the monomeric unit (n) and the cavity size CDs are classified as α (n = 6), β (n = 7), γ (n = 8) *etc.* The larger cavity of γ-CD permits formation of higher order complexes and often results in very different guest properties [21,22]. Binding strength of the encapsulated guest with CD owes its origin to (i) enthalpic contribution due to location of guest molecule in the hydrophobic cavity, (ii) hydrogen bonding possibility and (iii) entropic contribution from the release of high energy "hot-water" molecules from its cavity [23].

In our investigation, we demonstrate that the Stokes shift of DSS shows a linear dependency with solvent polarity, which is further verified by computational study. Hence, DSS is shown to be a well-suited solvatochromic fluorescent probe for determination of the micro-polarity inside macrocyclic cavity. We have found that the polarity inside β-CD cavity is very similar to a 1:1 mixture of methanol and water. We have also shown that the pH of the surrounding medium is quite essential for understanding and interpreting the photophysics of DSS. Moreover, encapsulation of DSS by CD causes a significant shift in the acid dissociation constant (*vide supra*), which results in a significant modulation of ground and excited-state properties. Such pH dependent complexation study of ICT dye with CD as host is rare, and a good care need to be taken while explaining the photophysics of the encapsulated dye. Time-resolved fluorescence studies further reveal protection of DSS from the bulk solvent as evident from enhancement in fluorescence lifetime.

2. Experimental

2.1. Materials

Dapoxyl sodium sulfonate (DSS) was purchased from Invitrogen (USA), α and β cyclodextrin (α-CD and β-CD) were purchased from Spectrochem (India), NaOH and HCl were purchased from SD-Fine (India) and Rankem (India), respectively. All the chemicals were used as received without any further purification. The solvents used for spectroscopic measurements were of spectroscopic grade; methanol and acetonitrile were purchased from Sigma–Aldrich (USA), acetone, benzene, chloroform and *n*-hexane were purchased from Sisco Research Laboratories (India) and no further purification was performed. Water used was of Mili-Q grade and ethanol was used after drying over magnesium metal overnight, followed by distillation under nitrogen atmosphere.

2.2. Steady state spectroscopic measurement

Steady state absorption measurements were done with Shimadzu UV-spectrophotometer using 10 mm path length quartz cuvettes. All the steady state fluorescence measurements were carried out by HORIBA Jobin Yvon Fluorimax-4 Fluorimeter. A 5 μM solution of DSS was taken for all the measurements to keep the absorption value minimum as to avoid inner filter effect. The experiments were carried out at pH 2.0, 4.0, 7.0 and 9.0, where the pH was controlled by using HCl and NaOH solutions. All the CD stock solutions were prepared depending on their water solubilities. Fluorescence spectra were recorded by 10 mm path length quartz cuvette in the region from 355 nm to 680 nm range by exciting at 340 nm with both excitation and emission slits operated with a width of 2 nm. All the experiments were carried out at room temperature.

2.3. Time resolved fluorescence measurement

Time resolved fluorescence measurements were performed using a time-correlated single photon counting (TCSPC) setup and using pulse diode laser for excitation (λ_{ex} = 375 nm) with full width and half maxima (fwhm) 167 ps and target count 10,000. The emission polarizer was positioned at a magic angle (54.75°) polarization with respect to excitation polarizer. The fitting was done by mono and bi-exponential fitting functions by iterative deconvolution method using software DAS v6.2. Following type of fitting function was used.

$$\frac{I(t)}{I(0)} = \sum a_i \exp(-t/\tau_i)$$

where $I(t)$ and $I(0)$ are the fluorescence intensity at time t and 0, t is time and a_i and τ_i are the contributing amplitude and corresponding lifetime. All the measurements were done at room temperature.